

In the Claims

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing. This listing of claims will replace all prior versions and listings of claims in the application:

1. (currently amended) Isolated RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of ~~an~~ the mRNA to which it corresponds.
2. (previously presented) Isolated RNA of claim 1 that comprises a terminal 3' hydroxyl group.
3. (currently amended) Isolated RNA of claim 1 which is chemically synthesized RNA ~~or an analog of a naturally occurring RNA.~~
4. (currently amended) An analog of isolated RNA of claim 1, wherein the analog differs from the RNA of claim 1 by the addition, ~~deletion~~, substitution or alteration of one or more nucleotides, wherein the one or more nucleotides added, substituted or altered is a non-naturally occurring nucleotide or deoxyribonucleotide.
5. (currently amended) Isolated RNA of from about 21 to about 23 nucleotides that has sequence correspondence to a gene and inactivates a the corresponding gene by transcriptional silencing.
6. (withdrawn) A soluble extract that mediates RNA interference.
7. (withdrawn) The soluble extract of Claim 6, wherein the extract is derived from *Drosophila* embryos.

8. (withdrawn) The soluble extract of Claim 7 wherein the extract is derived from syncytial blastoderm *Drosophila* embryos.
9. (withdrawn) A method of producing RNA of from about 21 to about 23 nucleotides in length comprising:
 - (a) combining double-stranded RNA with a soluble extract that mediates RNA interference, thereby producing a combination; and
 - (b) maintaining the combination of a) under conditions in which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides in length.
10. (withdrawn) The method of Claim 9, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.
11. (withdrawn) The method of Claim 9 further comprising isolating the RNA of from about 21 to about 23 nucleotides from the combination.
12. (currently amended) RNA of about 21 to about 23 nucleotides produced by the method of ~~Claim 9~~ comprising:
 - (a) combining double-stranded RNA with a soluble extract that mediates RNA interference, thereby producing a combination; and
 - (b) maintaining the combination of a) under conditions in which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides in length.
13. (withdrawn) A method of producing RNA of from about 21 to about 23 nucleotides in length that mediates RNA interference of mRNA of a gene to be degraded, comprising:
 - (a) combining double-stranded RNA that corresponds to a sequence of the gene to be degraded with a soluble extract that mediates RNA interference, thereby producing a combination; and
 - (b) maintaining the combination of (a) under conditions under which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA of the gene to be degraded, thereby producing

RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA.

14. (withdrawn) The method of Claim 13, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.

15. (withdrawn) The method of Claim 13 further comprising isolating RNA of from about 21 to about 23 nucleotides from the combination.

16. (currently amended) Isolated RNA of from about 21 to about 23 nucleotides that mediates RNA interference of mRNA of a gene to be degraded produced by the method of Claim ~~15~~ comprising:

- (a) combining double-stranded RNA that corresponds to a sequence of the gene to be degraded with a soluble extract that mediates RNA interference, thereby producing a combination; and
- (b) maintaining the combination of (a) under conditions under which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA of the gene to be degraded, thereby producing RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA, and further comprising isolating RNA of from about 21 to about 23 nucleotides from the combination.

17. (withdrawn) A method of mediating RNA interference of mRNA of a gene in a cell or organism comprising:

- (a) introducing RNA of from about 21 to about 23 nucleotides which targets the mRNA of the gene for degradation into the cell or organism;
- (b) maintaining the cell or organism produced in (a) under conditions under which degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.

18. (withdrawn) The method of Claim 17 wherein the RNA of (a) is a chemically synthesized RNA or an analog of naturally occurring RNA.
19. (withdrawn) The method of Claim 17, wherein the gene encodes a cellular mRNA or a viral mRNA.
20. (withdrawn) A method of mediating RNA interference of mRNA of a gene in a cell or organism in which RNA interference occurs, comprising:
- (a) combining double-stranded RNA that corresponds to a sequence of the gene with a soluble extract that mediates RNA interference, thereby producing a combination;
 - (b) maintaining the combination produced in (a) under conditions under which the double-stranded RNA is processed to RNA of from about 21 to about 23 nucleotides, thereby producing RNA of from about 21 to about 23 nucleotides;
 - (c) isolating RNA of from about 21 to about 23 nucleotides produced in (b);
 - (d) introducing RNA isolated in (c) into the cell or organism; and
 - (e) maintaining the cell or organism produced in (d) under conditions under which degradation of mRNA of the gene occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.
21. (withdrawn) The method of Claim 20, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.
22. (withdrawn) The method of Claim 20, wherein the RNA is isolated using gel electrophoresis.
23. (withdrawn) A method of mediating RNA interference of mRNA of a gene in a cell or organism in which RNA interference occurs, comprising: (a) introducing into the cell or organism RNA of from about 21 to about 23 nucleotides that mediates RNA interference of mRNA of the gene, thereby producing a cell or organism that contains the RNA and (b)

maintaining the cell or organism that contains the RNA under conditions under which RNA interference occurs, thereby mediating RNA interference of mRNA of the gene in the cell or organism.

24. (withdrawn) The method of claim 23, wherein the RNA of from about 21 to about 23 nucleotides is chemically synthesized RNA or an analog of RNA that mediates RNA interference.

25. (withdrawn) The method of Claim 23, wherein the gene encodes a cellular mRNA or a viral mRNA.

26. (withdrawn) A knockdown cell or organism generated by the method of claim 23.

27. (withdrawn) The knockdown cell or organism of claim 26, wherein the cell or organism mimics a disease.

28. (withdrawn) A method of examining the function of a gene in a cell or organism comprising:

- (a) introducing RNA of from about 21 to about 23 nucleotides that targets mRNA of the gene for degradation into the cell or organism, thereby producing a test cell or test organism;
 - (b) maintaining the test cell or test organism under conditions under which degradation of mRNA of the gene occurs, thereby producing a test cell or test organism in which mRNA of the gene is degraded; and
 - (c) observing the phenotype of the test cell or test organism produced in (b) and, optionally, comparing the phenotype observed to that of an appropriate control cell or control organism, thereby providing information about the function of the gene.
29. (withdrawn) The method of Claim 28 wherein the RNA introduced in (a) is chemically synthesized or an analog of RNA that mediates RNA interference.

30. (withdrawn) A method of examining the function of a--gene in a cell or organism comprising
- (a) combining double-stranded RNA that corresponds to a sequence of the gene with a soluble extract that mediates RNA interference, thereby producing a combination;
 - (b) maintaining the combination produced in (a) under conditions under which the double- stranded RNA is processed to RNA of about 21 to about 23 nucleotides, whereby RNA of about 21 to about 23 nucleotides is produced;
 - (c) isolating RNA of about 21 to about 23 nucleotides produced in (b);
 - (d) introducing the RNA isolated in (c) into the cell or organism, thereby producing a test cell or test organism;
 - (e) maintaining the test cell or test organism under conditions under which degradation of mRNA of the gene occurs, thereby producing a test cell or test organism in which mRNA of the gene is degraded; and
 - (f) observing the phenotype of the test cell or test organism produced in (e) and, optionally, comparing the phenotype observed to that of an appropriate control, thereby providing information about the function of the gene.
31. (withdrawn) The method of claim 30, wherein the RNA comprises a terminal 3' hydroxyl group.
32. (withdrawn) The method of claim 30, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.
33. (withdrawn) The method of claim 30, wherein the RNA is isolated using gel electrophoresis.
34. (withdrawn) A composition comprising biochemical components of a *Drosophila* cell that process dsRNA to RNA of about 21 to about 23 nucleotides and a suitable carrier.

35. (withdrawn) A composition comprising biochemical components of a cell that target mRNA of a gene to be degraded by RNA of about 21 to about 23 nucleotides.
36. (withdrawn) A method of treating a disease or condition associated with the presence of a protein in an individual comprising administering to the individual RNA of from about 21 to about 23 nucleotides that targets the mRNA of the protein for degradation.
37. (withdrawn) The method of claim 36 wherein RNA of from about 21 to about 23 nucleotides is chemically synthesized or an analog of RNA that mediates RNA interference.
38. (withdrawn) A method of assessing whether an agent acts on a gene product comprising:
- (a) introducing RNA of from about 21 to about 23 nucleotides which targets the mRNA of the gene for degradation into a cell or organism;
 - (b) maintaining the cell or organism of (a) under conditions in which degradation of the mRNA occurs,
 - (c) introducing the agent into the cell or organism of (b); and
 - (d) determining whether the agent has an effect on the cell or organism, wherein if the agent has no effect on the cell or organism then the agent acts on the gene product or on a biological pathway that involves the gene product.
39. (withdrawn) The method of claim 38, wherein the RNA of from about 21 to about 23 nucleotides is chemically synthesized or an analog of RNA that mediates RNA interference.
40. (withdrawn) A method of assessing whether a gene product is a suitable target for drug discovery comprising:
- (a) introducing RNA of from about 21 to- about 23 nucleotides which targets the mRNA of the gene for degradation into a cell or organism;
 - (b) maintaining the cell or organism of (a) under conditions in which degradation of the mRNA occurs resulting in decreased expression of the gene; and

(c) determining the effect of the decreased expression of the gene on the cell or organism, wherein if decreased expression has an effect, then the gene product is a target for drug discovery.

41. (withdrawn) The method of claim 40, wherein the RNA of from about 21 to about 23 nucleotides is synthetic RNA or an analog of RNA that mediates RNA interference.

42. (withdrawn) A gene identified by the sequencing of endogenous 21 to 23 nucleotide RNA molecules that mediate RNA interference.

43. (currently amended) A pharmaceutical composition comprising RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of the mRNA to which it corresponds and an appropriate carrier.

44. (withdrawn) A method of producing knockdown cells, comprising introducing into cells in which a gene is to be knocked down RNA of about 21 to about 23 nt that targets the mRNA corresponding to the gene and maintaining the resulting cells under conditions under which RNAi occurs, resulting in degradation of the mRNA of the gene, thereby producing knockdown cells.

45. (withdrawn) The method of claim 44, wherein the RNA of about 21 to about 23 nucleotides is synthetic RNA or an analog of RNA that mediates RNA interference.

46. (withdrawn) A method of identifying target sites within mRNA that are efficiently cleaved by the RNAi process, comprising combining dsRNA corresponding to a sequence of a gene to be degraded, labeled mRNA corresponding to the gene and a soluble extract that mediates RNA interference, thereby producing a combination; maintaining the combination under conditions under which the dsRNA is degraded and identifying sites in the mRNA that are efficiently cleaved.

47. (withdrawn) A method of identifying 21-23 nt RNAs that efficiently mediate RNAi, wherein said 21-23 nt RNAs span the target sites identified within the mRNA by the method of claim 46.

48.-50. (canceled herewith).

51. (withdrawn) The method of Claim 17 wherein the RNA is introduced into the cell or organism by a recombinant DNA method.

52. (withdrawn) The method of Claim 51 wherein the RNA is introduced into the cell or organism as DNA which encodes the RNA.

53. (withdrawn) The method of Claim 52 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.

54. (withdrawn) The method of Claim 23 wherein the RNA is introduced into the cell or organism by a recombinant DNA method.

55. (withdrawn) The method of Claim 54 wherein the RNA is introduced into the cell or organism as DNA which encodes the RNA.

56. (withdrawn) The method of Claim 55 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.

57. (withdrawn) The method of Claim 28 wherein the RNA is introduced into the cell or organism by a recombinant DNA method.

58. (withdrawn) The method of Claim 57 wherein the RNA is introduced into the cell or organism as DNA which encodes the RNA.

59. (withdrawn) The method of Claim 58 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.
60. (withdrawn) The method of Claim 36 wherein the RNA is administered to the individual by a recombinant DNA method.
61. (withdrawn) The method of Claim 60 wherein the RNA is administered to the individual as DNA which encodes the RNA.
62. (withdrawn) The method of Claim 61 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.
63. (withdrawn) The method of Claim 38 wherein the RNA is introduced into the cell or organism by a recombinant DNA method.
64. (withdrawn) The method of Claim 63 wherein the RNA is introduced into the cell or organism as DNA which encodes the RNA.
65. (withdrawn) The method of Claim 64 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.
66. (withdrawn) The method of Claim 40 wherein the RNA is introduced into the cell or organism by a recombinant DNA method.
67. (withdrawn) The method of Claim 66 wherein the RNA is introduced into the cell or organism as DNA which encodes the RNA.
68. (withdrawn) The method of Claim 67 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.

69. (withdrawn) The method of Claim 44 wherein the RNA is introduced into the cell by a recombinant DNA method.

70. (withdrawn) The method of Claim 69 wherein the RNA is introduced into the cell as DNA which encodes the RNA.

71. (withdrawn) The method of Claim 70 wherein the RNA encoded by the DNA is processed to RNA segments of about 21 to about 23 nucleotides in length.

72. (currently amended) Isolated DNA comprising DNA encoding RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of the mRNA to which the segments correspond.

73. (currently amended) Isolated DNA comprising DNA encoding RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to a gene and inactivates a the corresponding gene by transcriptional silencing.

74. (currently amended) Isolated DNA comprising DNA encoding RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to an mRNA and mediates RNA interference of the mRNA of a gene.

75. (currently amended) Isolated DNA comprising DNA encoding RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to an mRNA and targets the mRNA of a protein for degradation.

76. (currently amended) Isolated double-stranded RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of an the mRNA to which it corresponds.
77. (previously presented) Isolated double-stranded RNA of claim 76 that comprises a terminal 3' hydroxyl group.
78. (currently amended) Isolated double-stranded RNA of claim 76 which is chemically synthesized RNA ~~or an analog of a naturally occurring RNA~~.
79. (currently amended) An analog of isolated double-stranded RNA of claim 76, wherein the analog differs from the double-stranded RNA of claim 76 by the addition, ~~deletion~~, substitution or alteration of one or more nucleotides, wherein the one or more nucleotides added, substituted or altered is a non-naturally occurring nucleotide or deoxyribonucleotide.
80. (currently amended) -Isolated double-stranded RNA of from about 21 to about 23 nucleotides that has sequence correspondence to a gene and inactivates-a the corresponding gene by transcriptional silencing.
81. (currently amended) A pharmaceutical composition comprising double-stranded RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of the mRNA to which it corresponds and an appropriate carrier.
82. (currently amended) Isolated DNA comprising DNA encoding double-stranded RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of the mRNA to which the segments correspond.

83. (currently amended) Isolated DNA comprising DNA encoding double-stranded RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to a gene and inactivates a the corresponding gene by transcriptional silencing.

84. (currently amended) Isolated DNA comprising DNA encoding double-stranded RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to an mRNA and mediates RNA interference of the mRNA of a gene.

85. (currently amended) Isolated DNA comprising DNA encoding double-stranded RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that have sequence correspondence to an mRNA and targets the mRNA of a protein for degradation.

86. (currently amended) Isolated RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of ~~an~~ the mRNA to which it corresponds, wherein the isolated RNA is obtained from double-stranded RNA that has been cleaved into fragments of about 21 to about 23 nucleotides.

87. (previously presented) Isolated RNA of claim 86 that comprises a terminal 3' hydroxyl group.

88. (currently amended) Isolated RNA of claim 86 which is chemically synthesized RNA ~~or an analog of a naturally occurring RNA.~~

89. (currently amended) An analog of isolated RNA of claim 86, wherein the analog differs from the RNA of claim 86 by the addition, ~~deletion~~, substitution or alteration of one or more nucleotides wherein the one or more nucleotides added, substituted or altered is a non-naturally occurring nucleotide or deoxyribonucleotide.

90. (currently amended) Isolated RNA of from about 21 to about 23 nucleotides that inactivates a corresponding gene by transcriptional silencing, wherein the isolated RNA is obtained from double-stranded RNA that has been cleaved into fragments of about 21 to about 23 nucleotides and that has sequence correspondence to the gene.

91. (currently amended) A pharmaceutical composition comprising RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference of ~~an~~ the mRNA to which it corresponds, wherein the isolated RNA is obtained from double-stranded RNA that has been cleaved into fragments of about 21 to about 23 nucleotides.

92. (currently amended) Isolated DNA comprising DNA encoding RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference by directing cleavage of the mRNA to which the RNA correspond.

93. (currently amended) Isolated DNA comprising DNA encoding RNA of from about 21 to about 23 nucleotides that has sequence correspondence to a gene and inactivates a the corresponding gene by transcriptional silencing.

94. (currently amended) Isolated DNA comprising DNA encoding RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and mediates RNA interference of the mRNA of a gene.

95. (currently amended) Isolated DNA comprising DNA encoding RNA of from about 21 to about 23 nucleotides that has sequence correspondence to an mRNA and targets the mRNA of a protein for degradation.

96. (withdrawn) A method of producing RNA of from about 21 to about 23 nucleotides in length comprising:

- (a) combining RNA with a soluble extract that mediates RNA interference, thereby producing a combination; and
- (b) maintaining the combination of a) under conditions in which the RNA is processed to RNA of from about 21 to about 23 nucleotides in length.

97. (withdrawn) The method of Claim 96, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos:

98. (withdrawn) The method of Claim 96 further comprising isolating the RNA of from about 21 to about 23 nucleotides from the combination.

99. (withdrawn) RNA of about 21 to about 23 nucleotides produced by the method of Claim 96.

100. (withdrawn) A method of producing RNA of from about 21 to about 23 nucleotides in length that mediates RNA interference of mRNA of a gene to be degraded, comprising:

- a) combining RNA that corresponds to a sequence of the gene to be degraded with a soluble extract that mediates RNA interference, thereby producing a combination; and
- b) maintaining the combination of (a) under conditions under which the RNA is processed to RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA of the gene to be degraded, thereby producing RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA.

101. (withdrawn) The method of Claim 100, wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.

102. (withdrawn) The method of Claim 100 further comprising isolating RNA of from about 21 to about 23 nucleotides from the combination.

103. (New) Isolated RNA of claim 1 which is an analog of a naturally occurring RNA.

104. (New) Isolated double-stranded RNA of claim 76 which is an analog of a naturally occurring RNA.
105. (New) Isolated RNA of claim 86 which is an analog of a naturally occurring RNA.
106. (New) Isolated RNA of any one of claims 1, 43, 72, 74, 75, 76, 81, 82, 84, 85, 86, 91, 92, 94, and 95 wherein the isolated RNA is complementary to the mRNA.
107. (New) Isolated RNA of any one of claims 5, 73, 80, 83, 90, and 93 wherein the isolated RNA is complementary to the gene.
108. (New) Isolated RNA of any one of claims 1, 5, 12, 16, 43, 72, 73, 74, 75, 76, 80, 81, 82, 83, 84, 85, 86, 90, 91, 92, 93, 94, and 95 wherein the mRNA is human mRNA.
109. (New) Isolated RNA of any one of claims 1, 5, 12, 16, 43, 72, 73, 74, 75, 76, 80, 81, 82, 83, 84, 85, 86, 90, 91, 92, 93, 94, and 95 wherein the mRNA is mammalian mRNA.